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Control of Cellular Influx in Lung and Its Role in Pulmonary Toxicology

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The pulmonary influx of cytotoxic inflammatory cells, normally, in response to external toxins, is now thought to be etiologic in many of the disease syndromes of man, such as bronchitis and emphysema. Many types of effector inflammatory cells are involved, e.g., eosinophils, neutrophils, T-lymphocytes, monocytes. The diseases are characterized either by tissue destruction or by tissue hyperplasia. Agents which initiate the influx and cytotoxic secretions by these cells are legion and in general are not cell-specific. They include agents, such as phorbol esters, formyl peptides-complement fragments, elastin fragments, fatty acids (leukotrienes) as well as many uncharacterized excretions of inflammatory cells themselves, which react with specific receptors on the inflammatory cells, and secreted proteins such as fibronectin. Other agents, such as linoleic acid, digitonin and hydroxy fatty acids which are not bound by specific receptors also activate motility of inflammatory cells. The precise role of the above multiple cytotoxins in specific cellular fluxes in most pulmonary disease remains undefined.

Similarly, the mechanism of cytotoxicity used by specific invading cells in specific pulmonary syndromes remains unclear. In general, macrophages are thought to destroy using specific proteases, neutrophils use oxidant radicals and proteases and eosinophils use basic surface active peptides. T-cells kill by unknown mechanisms. However, in specific clinical syndromes, it is usually not clear which cell is the cytotoxic culprit, nor is the mechanism of destruction usually known.

The specific cellular influx and efflux of inflammatory cells into specific components of the mammalian lung is a major characteristic of several pulmonary toxic syndromes. This is also true, of course, for most pulmonary clinical syndromes (e.g., bronchitis, emphysema, cigarette smoke poisoning, acute respiratory distress syndromes) or the many granulomatous syndromes (eosinophilic granuloma, Wegner's granuloma, Boeck's sarcoid) observed in man. Each of these syndromes is characterized by influx and accumulation of specific inflammatory cells into specific pulmonary compartments.

There is now considerable information concerning the nonspecific influx of inflammatory cells into damaged tissues. However, little understanding concerning specific cellular influxes is available, and essentially no data are available concerning cellular efflux from healing tissues. An understanding of the clinical syndromes listed above in which the residence time of specific inflammatory cells in lung can be very long—of the order of years—is crucial to establish therapeutic or etiologic approaches to these diseases.

Multiple substances, whether inhaled or injected intratracheally or intravenously, will cause the accumulation of various inflammatory cells in various areas of the mammalian lung. For example, intratracheal injection of cotton extract, various other phenols, irritant substances or various gases will cause a rapid influx (4 hr) of polymorphonuclear leukocytes (PMNL) into airways (*1*). Similarly, intravenous injection of Freund's adjuvant, silica or aluminium silicate particles or inhalation of cigarette smoke, ozone or elevated concentrations of oxygen (*2,3*) results in accumulation of PMNL acutely and of macrophages subsequently into alveolar and interstitial compartments. Intratracheal injection of α-quartz in germ-free rats causes a delayed accumulation (3 mo) of macrophages without a preceding influx of PMNL (*4*).

In other species, however, e.g., fowl or amphibia, inflammatory cells do not invade the lung with exposure to intratracheal silica, intravenous Freund's adjuvant or hyperbaric oxygen (*5,6*). In fact, fowl lungs suffer no observable acute or chronic damage from hyperbaric oxygen or from intratracheal injection of silica or Freund's adjuvant (*6*). Thus, it is clear that different mechanisms exist, in various species, for controlling the efflux and accumulation of specific inflammatory cells in localized areas of the lung.

Since in these species those lungs are not damaged by known lung toxins, there is also no inflammatory cell influx, it is possible that much of the lung cell damage seen in mammalian lung diseases is the result of the cytotoxic capabilities of the inflammatory system itself.

The above observations, suggesting that the chronic lung damage seen clinically and after toxic exposures of

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mammals—but not nonmammalian species—may be the result of the destructive capabilities of the invading inflammatory cells themselves have raised three therapeutically important questions (*7*): What controls the influx and efflux of inflammatory cells into lung? What factors control the cytotoxic mechanisms of these cells? And most importantly, are these cytotoxic mechanisms of these inflammatory cells really responsible for the observed tissue destruction or hypertrophy seen in various clinical syndromes, e.g., emphysema, chronic bronchitis, asthma, interstitial fibrosis, and various chronic granulomatous syndromes, which appear to be clearly related to exposure to respirable toxins?

Control of Inflammatory Cell Motility

Specific Cytotaxins

Molecules of two major types (Table 1) are known to activate motility *in vitro* of inflammatory mammalian cells but not in other species (*5*). These two types are polypeptides and oxidized fatty acids (*8–10*).

Peptides. The peptides include synthetic peptides as well as many naturally occurring ones, e.g., various formylated peptides (*9*), specific fragments from complement (C_{5a}) (*11*), and helper peptides (*12*), α-globulins, collagen (*13*), elastin (*14*) and fibronectin (*15*). These peptides are also known to bind to different cell surface receptors present in varying amounts on granulocytes but not on lymphocytes (*16,17*). Binding of these receptors to their respective ligands is stoichiometrically related to degree of motility. Although optimal motility is observed when these peptides are presented to the cell in a concentration gradient (chemotaxis), these peptides also activate motility in the absence of a concentration gradient (chemokinesis). The magnitude of the nondirected migratory response *in vitro* depends on the concentration of cells, i.e., the freedom of the cells to move laterally, rather than vertically, through filters (*18*).

Chemical alteration of the peptide bond by *N*-alkylation of the synthetic chemotaxin, formyl-met-phe, causes a complete loss of binding to the formyl peptide receptor. However, some of the biologic activities, e.g., chemokinesis, inhibition of O_2^- production, of these peptide analogs remain (*19*). This indicates that the peptide bond itself is not necessary for all cell biologic functions of chemotactic peptides. Likewise, the clinical specificity of the receptor for the synthetic formyl peptides has been shown to be nonspecific. Pyrazolon anti-inflammagens, e.g., phenyl butazone, specifically inhibit binding of F-met-leu-phe to its receptor on human neutrophils (*20*).

Many uncharacterized biologic peptides with chemoattractant activities are also produced by various cells, e.g., lymphocytes, macrophages, mast cells and tumor cells in culture (*21–24*). It is not known if all these various cytotaxins have specific receptors, but it is clear that these agents possess marginal specificity for different cell types. Both polymorphonuclear leukocytes (PMNL), monocytes and alveolar or peritoneal macrophages are all stimulated by these peptides. However, it was recently shown that peptides containing desmosine (*14*), as well as desmosine itself are much more chemotactic for blood monocytes than for blood PMNL or alveolar macrophages. Fragments of fibronectin also appear to be somewhat specific cytotaxins for fibroblasts (*15*). An uncharacterized neutral substance of low molecular weight produced by human alveolar macrophages upon stimulation exhibit some specificity for neutrophils (*25*). The biologically derived acidic tetrapeptide has also been shown to be somewhat specific for motility of mammalian eosinophils (*21*), but it is not clear if this tetrapeptide is a major cytotaxin *in vivo*, since eosinophils also respond to many of the above peptides as well.

Fatty Acids. Two biologic unsaturated fatty acids,

Table 1. Cytotaxin specificity.

Cytotaxins	Activity of cells[a]			
	PMNL	Macrophages	Monocytes	Eosinophils
Peptides				
C_{5a}	+	+	+	+
Collagen fragments			+	
Elastin			+	
Eosinophil tetrapeptide	+			+
N-Formyl	+	+	+	+
N^1-Formyl N^2-alkyl	+	+		
Unsaturated fatty acids and lipids				
Arachidonic	+	+		
Linoleic		+		
Monohydroxy C_{20}	+	+		
Dihydroxy C_{20}	+			
Prostaglandin E_1	+			
Alveolar macrophage cytotaxin	+	+	+	+
Platelet activating factor			+	

[a] + = major cytotactic activity.

arachidonic and linoleic and their products, appear to be the major fatty acids involved in *in vivo* cytotaxis (*10, 26,27*) . Many of the oxidation products of arachidonic acid, e.g., prostaglandin E_1, the various monohydroperoxy and monohydroxy, i.e., 5-, 12- and 15-, eicosatetraenoic acids, and 5,12-dihydroxyeicosatetraenoic acid (*8,28*) as well as many uncharacterized photon-activated oxidation products of arachidonic acid (*10*) all exhibit chemoattractant properties *in vitro*. Arachidonic acid itself is also a powerful nonspecific attractant, even under conditions in which many of its metabolic pathways are inhibited. However, since arachidonic acid is so metabolically active, this acid does not usually accumulate in amounts sufficient to be physiologically active (*29*). Linoleic acid, however, does accumulate in considerable amounts, following injury, in body cavities, e.g., airways, pleura, and peritoneum (*27*). Various oxidation products of arachidonic acid, especially the hydroxy acids (*30*), also accumulate. In contrast to the peptides, however, these fatty acids are not bound by specific cell surface receptors on human PMNL (*26*). The mechanism by which these fatty acids activate motility is chemokinetic in type (*18*). Thus, chemotaxis which is the result of specific ligand-receptor interactions is usually not observed with the fatty acids.

The fatty acids, like the peptides, also possess cellular specificity. In fact, the only agent, thus far described that specifically attracts alveolar macrophages but not blood granulocytes is linoleic acid (*18*). This mechanism for linoleic acid attraction of macrophages is chemokinetic. Since the time course for influx and efflux of linoleic acid following injury in both airways of rabbits and in pleura of rats (*26*) closely parallels the influx and efflux of macrophages, it is likely that this chemokinetic mechanism which is stimulated by linoleic acid is operative in macrophage motility *in vivo*.

The source of this extravascular linoleic acid is probably the resident macrophages themselves. Stimulation of the isolated resident alveolar or pleural macrophages *in vitro* by agents such as zymosan or silica particles, phorbol esters, or calcium ionophores, causes a massive efflux of linoleic and other fatty acids from these cells. These fatty acids are derived almost exclusively from the phosphatidylcholines within these cells (*26*). Since essentially no arachidonic acid is liberated from these cells and since much of the secreted chemoattractant activities in these extracellular lavages can be accounted for as linoleic acid, it is likely that resident extravascular macrophages account, at least in part, for the accumulation of the cell-specific cytotaxin, linoleic acid, in injured extravascular spaces in mammals.

The above concept of the role of the secretions of resident inflammatory cells in extravascular spaces of mammals is supported by recent observations on other species. Essentially, no macrophages can be removed by lung lavage from fowl (*6*) (chickens) or amphibia (bull frogs) (*5*). These species also do not accumulate pulmonary inflammatory cells when exposed to hyperbaric oxygen or intratracheal respirable silica particles; nor does linoleic acid accumulate after exposure in airways of these two species; nor do these species suffer the hemorrhagic edematous lung damage seen in mammals exposed to hyperbaric oxygen (*5*). Thus, these observations on other species support the contention that the massive influx of inflammatory cells seen in mammalian lung, following exposure to oxidant or other types of toxins, is specifically involved in the resultant mammalian tissue damage.

Summary

It now appears that many inflammatory cells, as well as most extracellular structures (elastin, collagen and other secretory proteins) are the source of the active fatty acids or peptides, which can attract inflammatory cells, including fibroblasts, either specifically or nonspecifically. The peptide fragments specifically attract PMNL eosinophils or monocytes; the lipid fragments activate resident extravascular macrophages. The source of these biologically active products of proteases or phospholipases in largely the inflammatory cells themselves.

Species which do not accumulate resident macrophages in alveoli, e.g., fowl or amphibia, when exposed to mammalian toxins such as O_2 or silica, do not develop tissue damage upon exposure.

Inflammatory Cell Cytotoxicity

The above considerations implicating the inflammatory system as etiologic in many environmental toxic syndromes make it imperative that we understand how the cytotoxic potential of the inflammatory system is controlled. Three major cytotoxic systems are known, i.e., neutral proteases (*31*), neutral phospholipases and their inhibitors (*26,32*) and the oxidant–radical-producing system (*33*). The latter system is localized primarily in blood granulocytes, whereas the two hydrolase systems are localized primarily in macrophages.

The immune system, of course, with its vast array of monoclonal specificity is primarily concerned with focusing the above cytotoxic systems. With its capacity to recognize specific cells, either foreign or domestic, it directs the cytotoxic cells to their assigned target. This immunologic recognition and direction is beyond the scope of this review and is not further discussed. It is clear, however, that the cytotoxic system can work equally well either on its own direction or as directed by the immune system. This discussion is concerned only with the self-directed cytotoxic mechanisms of granulocyte and macrophages.

Oxidant Radical Production

Oxygen-derived radicals produce hydroxylated purines or pyrimidines, lipid hydroperoxides, auto-oxidized

products of thiols or amines, as well as depolymerized polysaccharides (*33*). Under ambient conditions, the major cellular source of extracellular oxidants (O_2^-, H_2O_2, $OH^\cdot$) is PMNL. Macrophages and fibroblast secrete considerably lesser amounts (*34*). Under hyperoxic conditions, however, it is likely that oxidant radical production (by cytoplasmic oxidases, mitochondrial oxidases and microsomal oxidases) by most cells can increase to a degree that the intracellular scavengers (superoxide dismutase, glutathione reductase, catalase, prostaglandin and thromboxane synthetase and lipoxygenase) as well as the extracellular scavenger, superoxide dismutase (*35*), fail to control intracellular concentrations. This leads to the various types of cell damage as indicated above, with the result that all cells can be potentially destroyed by internal oxidants.

At ambient conditions, cytotoxic destruction by extracellular oxidants is most likely the work of PMNL. The control of oxidant secretion in PMNL remains poorly understood. Almost any surface-active molecule, e.g., hydrophobic peptides, unsaturated fatty acids, digitonin, phorbol esters, calcium ionophores, cytochalasins, etc., will all activate this process (*36,37*). O_2^- appears to be the major excretory product (*38*), but the toxic species is the hydroxyl radical (*39*). The only known way to inhibit this process is to remove a bound protease from these cells, either by extensive washing, or by displacement of the protease by small amounts of formylated peptides or cytotactic fatty acids or by inhibition of the protease by addition of protease inhibitors (*40*). However, once the secretion of radicals is fully activated, e.g., by ionophores, digitonin or phorbol esters, the protease inhibitors are ineffective. Also, there are no other known inhibitors. Secretion of destructive oxidant radicals by leukocytes, therefore, is a process which is readily triggered by very low concentrations of innumerable small molecules, but in contrast to the other inflammatory systems, there is no known mechanism to inhibit this oxidant radical system, once it is fully activated.

Phospholipases

There is no evidence that inflammatory cells secrete phospholipases for cytotoxic purposes. However, as indicated above, macrophages, when stimulated, activate a phospholipase A_2 which liberates biologically active fatty acids, e.g., linoleic acid. Similarly, activation of a phospholipase in neutrophils results in the liberation of hydroxy fatty acids, e.g., 5-hydroxyeicosatetraenoic and 5,12-dihydroxyeicosatetraenoic. Thus, the function of these activatable phospholipases is to produce products which attract specific inflammatory cells.

Neutral Proteases

Neutral proteases, secreted both by activated macrophages and by PMNL may be directly involved in tissue destruction.

The elastase of PMNL and macrophages (*41*) destroy extracellular structures (elastin) (*42*) whereas the neutral proteases of macrophages are directly cytotoxic (*31*). The involvement of inflammatory proteases, e.g., neutrophil elastase in clinical lung destruction (emphysema) is now generally accepted (*43*). Toxic oxidants such as cigarette smoke, or oxidant radicals, produced by activated neutrophils, directly inactivate the protease inhibitor, α_1-trypsin inhibitor (*5*). This destruction of the natural inhibitor of the elastase results in the excessive destruction of elastin by neutrophil elastase with the development of pulmonary emphysema. It is not clear if hydrolytic enzymes of macrophages are also involved in pulmonary destruction. These macrophage cytotoxic proteases may be involved in tumor surveillance (*31*) or in autoimmune mechanisms which result in destruction of normal cells (*44*). It is possible that pulmonary tissue-specific antigen is involved in some of the many granulomatous pulmonary syndromes seen in man in which macrophages are the effector cells present. Example of such syndromes involving tissue specific antigens in other organs include multiple sclerosis (*45*), thyroiditis (*46*) and chronic bowel disease (*29*). In these various syndromes, it is unclear whether killer T-lymphocytes or macrophages are the cytotoxic effector cells and also if neutral hydrolases or oxidant radicals are the cytotoxic molecules.

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